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US AIR FORCE FUNDED STUDY OF HYDRAZINE METABOLISM AND TOXICITY

DR. J A TIMBRELL, TOXICOLOGY UNIT, SCHOOL OF PHARMACY,
UNIVERITY OF LONDON.

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The research project studying hydrazine in rats has revealed the following:
1. The uptake of hydrazine into the liver may be a saturable process.
2. After a hepatotoxic dose of hydrazine the concentration in the liver is about 0.2mM.
3. Some hydrazine remains in the liver 24 hours after a single dose at a level higher than the plasma level.
4. At the highest dose level (81mg/kg) rats lost weight over the following 4 days and liver weight was decreased. At a dose of 27mg/kg, the liver weight was elevated 4 days after the single dose.
5. The rats given the highest dose still showed fatty liver 4 days after dosing. Rats given lower doses also showed some vacuolation in hepatocytes.
6. Determination of hydrazine and acetylhydrazine in urine after various single doses showed a dose dependent decrease in acetylation.

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7. NMR revealed a number of metabolites: unchanged hydrazine, acetyl and diacetylhydrazine, hydrazones with pyruvate and 2-oxoglutarate, urea and ammonia.
8. Studies in hepatocytes have shown that hydrazine is cytotoxic at concentrations of 16mM and above.
9. Hepatocyte studies have also shown that 8mM hydrazine depletes ATP and glutathione.
10. Preliminary studies in microsomes have shown that hydrazine is metabolised by a cytochrome P450 dependent system. Chemical breakdown and other enzymic breakdown are also involved however.

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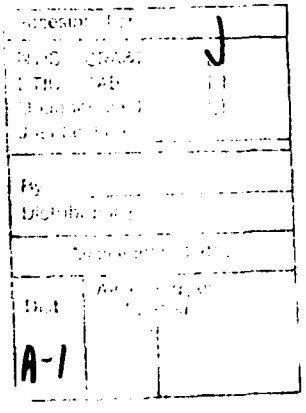
SUMMARY Studies in hydrazine in rats

The research project has revealed the following:

1) The uptake of hydrazine into the liver may be a saturable process;
2) After a hepatotoxic dose of hydrazine the concentration in the liver is about 0.2mM;
3) Some hydrazine remains in the liver 24 hours after a single dose at a level higher than the plasma level;
4) At the highest dose level (81mg/kg) rats lost weight over the following 4 days and liver weight was decreased. At a dose of 27mg/kg, the liver weight was elevated 4 days after the single dose;
5) The rats given the highest dose still showed fatty liver 4 days after dosing. Rats given lower doses also showed some vacuolation in hepatocytes;
6) Determination of hydrazine and acetylhydrazine in urine after various single doses showed a dose dependent decrease in acetylation;
7) NMR revealed a number of metabolites: unchanged hydrazine, acetyl and diacetylhydrazine, hydrazones with pyruvate and 2-oxoglutarate, urea and ammonia;
8) Studies in hepatocytes have shown that hydrazine is cytotoxic at concentrations of 16mM and above;
9) Hepatocyte studies have also shown that 8mM hydrazine depletes ATP and glutathione; AND
10) Preliminary studies in microsomes have shown that hydrazine is metabolised by a cytochrome P450 dependent system. Chemical breakdown and other enzymic breakdown are also involved however. (KR) ←



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INTRODUCTION

Hydrazine is an important chemical substance being used extensively in industry, as a rocket fuel and it is a metabolite of two widely used drugs (Timbrell and Harland, 1979; Blair et al, 1984).
We have previously studied hydrazine toxicity and metabolism (Wright and Timbrell, 1978; Timbrell et al, 1982), most recently using the technique of high resolution NMR (Sanins et al, 1988).
The object of this project is to study the metabolism and disposition of hydrazine in vivo and in vitro in relation to the hepatotoxicity.
The funding for the project supports a graduate research student who is working full time on the project. A graduate research student and a postdoctoral research worker, funded from other sources have also contributed to some of the work in this report. The graduate research student is in her final year and the postdoctoral research worker will be finishing in one month.

The details and results of the research on hydrazine metabolism and toxicity in this report may be divided into 3 areas:

1. In vivo studies.
The disposition of hydrazine after various doses. Metabolic information gained using NMR.

2. Studies on cytotoxicity in hepatocytes.

3. Preliminary studies on hydrazine metabolism in subcellular fractions.

This report will concentrate mainly on the first of these areas.

IN VIVO STUDIES

A study was carried out in rats to determine the disposition of hydrazine after different doses. This is important as the metabolism and/or disposition of hydrazine may vary with dose and hence the toxicity may depend on this. It was also important to determine the plasma and liver concentrations after exposure to hydrazine for reference for future in vitro studies.
The study was divided into two parts.

i) to determine the plasma and liver concentrations at short time intervals after various doses of hydrazine.
ii) to determine the urinary metabolites after the same doses of hydrazine and to examine the liver pathology 4 days after exposure.

EXPERIMENTAL DETAILS

EXPERIMENT 1. Determination of time course of plasma and liver hydrazine levels.

12 Rats (male Sprague Dawley, approx 200g) were used at each dose, 3 rats per time point, 4 time points. Each rat was weighed immediately before dosing. Rats were dosed orally with hydrazine hydrate in distilled water at staggered intervals starting at 11.00 am. Note, rats had no food for previous 24 hours.
The doses administered were 4.7, 14.1, 42.2 and 126 mg/kg hydrazine hydrate equivalent to 3, 9, 27 and 81 mg/kg hydrazine. The dose solution was administered by stomach tube. Each dose group (12 rats) was studied on a separate day. Animals were killed by exsanguination from the vena cava under ether anaesthesia at the following time points: 10 min, 30 min, 90 min and 270 min after dosing.

Procedure at each time point:

At the specified time after dosing each rat was anaesthetised with ether, blood was taken from the vena cava into a heparinised syringe then into a heparinised tube, centrifuged (2000rpm) and plasma removed. The liver was removed from the animal, weighed and 5g homogenised in 20ml methanol/water (50/50). The homogenate was centrifuged and the supernatant was removed. The remainder of the liver was frozen at -80°C. The internal standard for the gc/ms assay was added to aliquots of supernatant and plasma immediately as described below.
The same procedure was carried out for each rat for each dose and time point. Control plasma samples were obtained from the control rats in experiment 2.

EXPERIMENT 2. Determination of effect of various oral doses of hydrazine on body weights, organ weights and liver pathology.

Day 1. 15 rats (male Sprague Dawley, approx. 220g) were weighed and dosed orally in groups of 3 at intervals. Rats had been starved for the previous 24 hours. Dosing was with hydrazine hydrate in water (0, 3, 9, 27, 81 mg/kg hydrazine as free base). After dosing rats were placed in metabolism cages and urine was collected daily over ice for 4 days.

Day 2. Rats weighed ,urine collected and volume measured and water intake recorded.

Day 3. Rats weighed ,urine collected and volume measured and water intake recorded.

Day 4. Rats weighed ,urine collected and volume measured and water intake recorded.

Day 5. Rats weighed, urine collected and volume measured. Water intake recorded. Each rat was anaesthetised with ether, blood was taken from the vena cava in controls and the highest dose group for subsequent analysis. Major organs (liver, testes, heart, kidneys, spleen) were taken from all rats, weighed and kept for histology. Section of liver removed for histology. Blood centrifuged for plasma. 5g of liver homogenised from control and highest dosed rats, remainder frozen.
Organs were fixed in buffered formalin. Liver sections were embedded in paraffin wax for sectioning for subsequent staining with haemotoxylin and eosin and frozen sections were cut for Oil Red O staining.
All urine and remaining plasma and liver samples have been kept frozen at -80°C.

Procedure for assay of hydrazine in plasma and liver:

0.1ml of internal standard solution, (^{15}N hydrazine sulphate; 100 M) was added to 0.9ml of plasma sample or 4.9ml of liver supernatant. 0.2ml of 1M HCl and 2.3ml of saturated ammonium sulphate solution added to plasma or liver supernatant sample in screw top tube. Sample vortexed (30 secs) and centrifuged (20 min; 2500 g). Supernatant removed and an equal volume of citrate buffer (0.5M; pH 6) added. Mixture vortexed and allowed to stand. 20 ml dichloromethane added and mixture vortexed. Layers separated by centrifugation. Aqueous layer removed and 0.1ml penta-fluorobenzaldehyde solution in methanol (1g/5ml) added. Mixture vortexed and left to stand for 30 min. Mixture extracted with chloroform (5ml) and chloroform extract separated and used for gc/ms assay.
A standard curve was also prepared using spiked samples.

Hydrazine concentration was determined by gas chromatography/mass spectrometry with selected ion monitoring for the penta-fluorobenzaldehyde azine molecular ion. The ratio of ^{14}N to ^{15}N azine was determined and used to calculate the concentration of ^{14}N hydrazine from the standard curve.

Hydrazine and acetylhydrazine were determined in urine by gas chromatography by the following method.

Urine adjusted to pH 5 was pre-extracted with methylene chloride. An equal volume of citrate buffer (pH 6) was added, the internal standard, the penta-fluorobenzyl-penta-fluorophenylhydrazone plus pentafluorobenzaldehyde. After 30 minutes reaction time the mixture was extracted with methylene chloride. The extract was then reduced to dryness and taken up

in ethyl acetate. This was used for gas chromatographic determination.

At the present time only the first 24 hour urine collection has been analysed for these two metabolites.

STUDIES USING ^{15}N HYDRAZINE.

$^{15}N_2$ Hydrazine (98.6 % g atom) was obtained from MSD Isotopes Ltd., Montreal, Canada. Other standards were obtained from Sigma Chemical Co, or Aldrich Chemical Co. THOPC was synthesised by reacting equimolar amounts of 2-oxoglutarate and hydrazine hydrate.
Male, Sprague-Dawley rats (200 g) were dosed intraperitoneally with ^{15}N hydrazine hydrate (1.88 mmol or 2.5mmol/kg) and urine was collected over ice for 24 hours.
For ^{15}N NMR urine samples were lyophilized so as to reduce the volume to 25%. $^{2}H_2O$ (D_2O; 5-20% v/v) was added for a field frequency lock.
NMR conditions were as follows: samples were analysed in 10 or 15mm tubes in a Bruker WH400 NMR spectrometer. Chemical shifts were referenced to ^{15}N ammonium nitrate solution at 0 ppm at40.56MHz in an internal concentric NMR tube. Exponential functions corresponding to line broadenings of 10-30 Hz were applied prior to Fourier transformation to improve signal to noise ratios.
Liver and blood samples were also removed from the animals at termination of the experiment. These were then assayed for hydrazine essentially as described above by gas chromatography/mass spectrometry except that ^{14}N hydrazine was used as internal standard.

STUDIES IN HEPATOCYTES

Rat hepatocytes have been used to study the toxicity of hydrazine in vitro. Male Sprague-Dawley rats (250g) were used. Hepatocytes were isolated from liver by the standard collagenase perfusion method (Moldeus et al, 1978). They were then incubated in Krebs-Henseleit buffer in an atmosphere of 95% O_2/5% CO_2 at a cell density of 2.25 x 10^6 for periods up to 4 hours in the presence of various concentrations of hydrazine. Aliquots of cells were taken at hourly intervals from the incubation buffer. Cells were gently sedimented and resuspended in fresh buffer. Cytotoxicity was determined by the Trypan Blue exclusion method. This relies on the fact that damaged cells will take up the dye (0.2% Trypan Blue in 0.9% saline) but viable cells will not. Therefore simple counting of stained cells indicates the numbers of dead cells. The cells in the remainder of the aliquot were rapidly vortexed in 10% trichloroacetic acid for assay for ATP.
As well as cytotoxicity, the levels of ATP and glutathione were measured, also indicators of cell viability.
ATP was measured by a luciferase-linked bioluminescence method

(Stanley and Williams 1969) in trichloroacetic acid extracts from hepatocytes. Hydrazine did not interfere with this assay.

Glutathione was measured by the method of Ellman (1959) as hydrazine was found to interfere with the more sensitive method of Hissin and Hilf. However Ellmans method was adequate for the determinations carried out.

The studies carried out so far have been a study of hydrazine cytotoxicity, with measurement of glutathione and ATP over the concentration range 0-20mM.

PRELIMINARY STUDIES IN MICROSOMES

Preliminary studies have been carried out to determine the metabolism of hydrazine in subcellular fractions. Thus far hydrazine disappearance in microsomal suspensions has been examined. Hydrazine was measured by a spectrophotometric method involving reaction of hydrazine with dimethylaminobenzaldehyde. The protocol for this assay is shown in Figure 10. This method was utilised because although we have a gas chromatographic method for determination of hydrazine in urine this was too time consuming for use with the microsomal incubations. For the purposes of measuring the disappearance of hydrazine the spectrophotometric assay is adequate. Microsomes were prepared from male Sprague-Dawley rats (250 g) by the standard technique using ultracentrifugation. Microsomes were incubated for 60 minutes with an NADPH generating system and hydrazine at a concentration of 2mM and a protein concentration of 4 mg/ml. The effect of replacing oxygen with nitrogen, a carbon monoxide/oxygen atmosphere and the presecence of the enzyme inhibitors piperonyl butoxide, metyrapone and methimazole was investigated. Aliquots of the reaction mixture were removed at various times and in these the reaction was stopped by the addition of trichloroacetic acid. The concentration of hydrazine remaining in the incubation mixture was then measured by the spectrophotometric method.

RESULTS AND DISCUSSION

The results from Experiment 1 are shown in Tables 1 and 2 and the data in Table 1 is plotted in Figures 1 and 2. The plasma levels show the expected increase with dose and approximately similar rates of decline except for the highest dose. Thedata in Table 1 shows that the maximum liver concentration of hydrazine is about 0.2 mM after the highest and toxic dose. This is important with reference to the concentration of hydrazine to which hepatocytes are exposed (see below). The data in Table 1 also indicates that the liver level does not increase proportionately with increasing dose. This is readily seen in Figure 2. At the lowest dose about 1% of the dose is present in the liver. At the highest dose this is 0.045%. Clearly uptake into the liver is not a constant fraction of the dose. In contrast the maximum plasma levels show an approximately proportionate increase (Table 1). The ratio of plasma to liver reflects these changes with a ratio greater than 1 at higher doses (Table 2).
These data suggest that transport of hydrazine into the liver is saturable and does not occur by simple passive diffusion in which case approximately equal concentrations would be reached. Clearly from the plasma liver ratio this is not the case and it is also evident from Figure 2. This however is based on the assumption of the concentration of hydrazine being distributed equally throughout the mass of liver tissue.
Of relevance to this discussion is the data in Table 6. From this data it is clear that some hydrazine remains in the liver 24 hours after dosing. Thus the plasma to liver ratio is 0.17 at this time point. This is consistent with transport of hydrazine into the liver not being simple passive diffusion as the ratio should be about 1 in that case. This sequestration of hydrazine in liver has important toxicological implications, suggesting that repeated exposure might lead to accumulation. This possibility is therefore currently being further investigated.
Also notable in Table 1 are the apparent increases in liver weights and body weights. A rapid increase in liver weight after hydrazine has been noted before (Timbrell et al, 1982) but is of unknown mechanism.

The results from Experiment 2 are shown in Tables 3, 4 and 5 and Figures 3 and 4. It can be seen from Table 3 and Figure 3 that apart from the highest dose group, the controls and all hydrazine dosed animals gained weight. The top dose group (81mg/kg) lost weight over the 4 days. Also the animals in the top dose group took in more water and excreted more urine in the first 24 hours than the controls or other dosed animals. When the organ weights are considered it can be seen (Table 4, Figure 4) that the liver weight in the group dosed with 27mg/kg hydrazine showed a significant increase in liver weight (expressed as a ratio with body weight). None of the other organ weights were significantly affected. The absolute liver

weights for the top dose group (81mg/kg) were in fact significantly reduced, as were the body weights for this group.

Pathology.

Liver sections were examined from rats in Experiment 2, in which livers were removed from rats 4 days after various oral doses of hydrazine.

Histological slides were examined blind. Sections stained with haemotoxylin and eosin from rats dosed with 9 and 27 mg/kg hydrazine showed a slight amount of vacuolation which was not obviously zonal. Sections from all the rats given the highest dose of hydrazine (81 mg/kg) clearly had intracellular droplets. The droplets were not clearly zonal in distribution, being throughout the lobule in one rat, but more localised around the vessels, both portal tract and central vein, in the sections from the other two rats. Only sections from two of the rats given the highest dose of hydrazine (81mg/kg) showed fatty deposits as indicated by Oil Red O staining. These fatty deposits were located mainly in the midzonal area of the lobule. Clearly the steatosis caused by hydrazine persists for at least 4 days after dosing in the top dose and there is histological evidence of pathological changes (vacuolation) at the lower doses of 9 and 27 mg/kg.

Metabolism

Measurement of hydrazine and acetylhydrazine by gas chromatography in the urine collected 0-24 hours after the various oral doses (3-81 mg/kg) of hydrazine revealed a dose dependent decrease in the excretion of both compounds (Table 5). That the acetylation of hydrazine to acetylhydrazine is a dose dependent process is not unexpected from previous studies with acetylhydrazine and isoniazid (Wright and Timbrell, 1978, Timbrell and Wright, 1979). The decrease in the excretion of hydrazine is more difficult to explain unles excretion is a dose dependent, saturable process. This again has important toxicological implications, with the possibility of accumulation in an animal exposed repeatedly to hydrazine. Alternatively the metabolism may change at higher doses to compensate. This is an area for further study.
We have previously used proton NMR of urine and tissue extracts to study hydrazine metabolism and toxicity (Sanins et al, 1988) and this had revealed that acetylhydrazine and diacetylhydrazine were both detectable in the urine and had also indicated a new metabolite, the cyclised hydrazone of hydrazine and 2-oxoglutarate (THOPC), (Fig. 5).
Examination of the ^{15}N NMR spectra revealed a number of resonances (Fig. 6,7). Some of these were identified by the addition of authentic standards. Thus it was clear that unchanged hydrazine, acetylhydrazine, diacetylhydrazine and the cyclised 2-oxoglutarate hydrazone, THOPC were present in urine. Also other peaks were detected which were not initially

identified. However with consideration of the spectra and the use of standards these were identified as ammonia and urea. Although a pure standard of the pyruvate hydrazone was not available when pyruvate and hydrazine were mixed in the NMR tube a resonance with the same chemical shift as that seen in urine was detected. The other resonances have so far not been identified.

Although resonances have been observed with similar chemical shift values to methylamine and monomethylhydrazine these have not been verified by gas chromatogaphy/mass spectrometry. Methylamine has indeed been detected by proton NMR and gas chromatography/mass spectrometry but it is not labelled with ^{15}N after the administration of ^{15}N hydrazine. The methylamine detected and quantitated in proton NMR spectra must therefore be endogenous,although the levels are raised by hydrazine.

The metabolic studies utilising NMR have revealed several new metabolites of hydrazine and clarified some uncertainties. Thus it is clear that unchanged hydrazine is excreted into the urine and is not only present as hydrazones. Similarly acetylhydrazine is present in the unchanged form and diacetylhydrazine is clearly seen to be present. Previously only indirect methods had been available to show that diacetylhydrazine existed as a urinary metabolite (Timbrell et al, 1977). The NMR technique has shown unequivocally that a cyclised hydrazone formed from hydrazine and 2-oxoglutarate is present in both liver and urine and that possibly a pyruvate hydrazone is also a metabolite.

Of particular interest however is the finding that ammonia and urea are urinary metabolites. These data taken together indicate that the N-N bond is split and that ^{15}N ammonia is incorporated into urea. The metabolic pathway involved has yet to be determined however.

Hepatocyte Studies

The studies with isolated hepatocytes have revealed that hydrazine shows a dose dependent cytotoxicity with concentrations between 12-16mM and above being cytotoxic in incubations over 4 hours (Fig. 8). The cytotoxicity became apparent at 3 hours after the start of exposure.

When ATP was measured this was significantly depleted by all concentrations of hydrazine (8-20mM) by 2 hours and within 1 hour by concentrations above 12mM (Fig. 9).

Glutathione was also found to be depleted by all the concentrations of hydrazine (8-20mM) by 3 hours and by 2 hours at the highest concentration (20mM) (Fig. 10).

Therefore it is clear that hydrazine is depleting both ATP and glutathione at a concentration (8mM) well below the cytotoxic concentration (16mM). Also the depletion of both ATP and glutathione occur before cytotoxicity is apparent. This indicates that ATP depletion especially may be involved in the mechanism of toxicity of hydrazine.

Another marker of cytotoxicity in hepatocytes, lactate dehydrogenase, is also currently being measured as an addition to Trypan Blue exclusion.
Studies in progress include use of cultured hepatocytes in which longer term exposure may be examined with more sensitive parameters of toxicity such as protein synthesis. Proton NMR is also being utilised in these studies.

Preliminary Microsomal Studies

The preliminary studies have revealed that hydrazine disappearance from a microsomal suspension is dependent on the presence of NADPH and oxygen. The requirement for cytochrome P450 is indicated by the inhibitory effect of carbon monoxide. (Table 7). Also the effect of microsomal enzyme inhibitors , piperonyl butoxide and metyrapone supports this. Therefore one can conclude that at least part of the disappearance of hydrazine is catalysed by cytochrome P450. However there is significant disappearance in the absence of NADPH and this seems to be very variable. This may be due to chemical breakdown or the action of other microsomal enzymes. The effect of methimazole, a microsomal amine oxidase inhibitor indicates other enzymes might be involved. So far however it has not been possible to derive kinetic parameters (Km and Vmax) for the enzyme catalysed disappearance of hydrazine. Therefore this is under further investigation in order to determine the source of the variation and to investigate the role of other enzymes.

LEGENDS TO FIGURES

Figure 1. Plasma hydrazine levels after various doses of hydrazine were administered to rats. Hydrazine determined by gas chromatography/mass spectrometry.

Figure 2. Liver hydrazine levels after various doses of hydrazine were administered to rats. Hydrazine determined by gas chromatography/mass spectrometry.

Figure 3. Effect of hydrazine on body weight of rats given a single dose of hydrazine on the first day (Monday).

Figure 4. Effect of hydrazine on organ weights of rats given a single dose of hydrazine on the first day (Monday). Weights are given relative to body weight.
LW: Liver wt; TW: Testis wt; KW: Kidney wt; SW: Spleen wt; HW: Heart wt.

Figure 5. Metabolism of hydrazine.

Figure 6. ^{15}N NMR spectrum (0-180ppm range) of urine from a rat dosed with ^{15}N labelled hydrazine (80mg/kg). Resonances are labelled. NHR represents the amido nitrogen, NH_2 represents the terminal nitrogen in acetylhydrazine.

Figure 7. ^{15}N NMR spectrum (0-360 ppm range) of urine from a rat dosed with ^{15}N labelled hydrazine (80mg/kg). Resonances are labelled with the relevant structure. R is H or CH_3CO.

Figure 8. Effect of hydrazine on hepatocyte viability. Hepatocytes were incubated with the following concentrations of hydrazine: ▲ 0mM; ◆ 8mM; ■ 12mM; ★ 16mM; ● 20mM. Asterisks denote a significant loss in viability.

Figure 9. Effect of hydrazine on hepatocyte ATP levels. Hepatocytes were incubated with the following concentrations of hydrazine: ▲ 0mM; ◆ 8mM; ■ 12mM; ★ 16mM; ● 20mM. Asterisks denote a significant drop in ATP level.

Figure 10. Effect of hydrazine on levels of reduced glutathione in hepatocytes. Hepatocytes were incubated with the following concentrations of hydrazine: ▲ 0mM; □ 8mM; ■ 12mM; △ 16mM; ● 20mM. Asterisks denote a significant fall in glutathione level.

Figure 11. Protocol for determination of hydrazine in microsomal incubations.

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Preece, N E, Nicholson, J K and Timbrell, J A. "The use of ^{15}N-NMR in the study of hydrazine metabolism and toxicity". Ninth International Meeting on NMR Spectroscopy. Warwick, July 1989.

Ghatineh, S and Timbrell, J A. "Study of hydrazine toxicity in isolated hepatocytes". V International Congress of Toxicology, Brighton, July 1989.

Dr. J A Timbrell visited Toxico Hazards Division, Wright-Patterson AFB, Dayton Ohio on 26-27th September 1989. Here he delivered a seminar on his work on hydrazine and met with many of the staff but especially with Ms George, Chief of the Biochemistry Branch. The subject of the discussions was mainly hydrazine toxicity.

ARTICLES RELATING TO RESEARCH: PUBLISHED, IN PRESS OR IN PREPARATION:

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* Arising from presentations at scientific meetings.

PARTICIPATING PROFESSIONALS:

Dr. J A Timbrell, Senior Lecturer in Toxicology, University of London. Permanent position.

Dr. N E Preece, postdoctoral research assistant, funded by Wellcome Trust. Grant terminates December 1989.

Ms S Ghatineh, graduate research student, funded by Science and Engineering Research Council and Schering Agrochemicals. Grant terminates August 1990.

Mr. A Jenner, graduate research student, funded by USAF, (EOARD). Grant terminates 1991.

TABLE 1.

TIME COURSE OF HYDRAZINE IN PLASMA AND LIVER.

DOSE 3mg/kg.

TIME	BODY WEIGHT	LIVER WT	LW/BW	PLASMA HYDRAZINE	LIVER HYDRAZINE
min	g	g		nmoles/ml	nmoles/g
10	188	5.58	0.030	22.3 ± 4.2	33.2 ± 23.8
30	200	6.09	0.030	13.0 ± 4.7	20.5 ± 3.6
90	200	6.25	0.031	10.7 ± 2.5	14.4 ± 1.9
270	207	7.2	0.035	6.3 ± 2.6	5.2 ± 0.5

DOSE 9mg/kg.

TIME	BODY WEIGHT	LIVER WT	LW/BW	PLASMA HYDRAZINE	LIVER HYDRAZINE
10	188	5.9	0.031	41.7 ± 29.9	135.0 ± 25.3
30	196	6.3	0.032	53.0 ± 5.1	138.3 ±171.3
90	193	6.4	0.033	43.2 ± 8.6	20.4 ± 13.4
270	201	6.89	0.034	12.0 ± 5.2	12.3 ± 11.4

DOSE 27mg/kg.

TIME	BODY WEIGHT	LIVER WT	LW/BW	PLASMA HYDRAZINE	LIVER HYDRAZINE
10	197	6.1	0.031	235.9 ± 237.5	161.4 ± 74.0
30	202	6.9	0.034	221.2 ± 163.0	80.1 ± 28.2
90	200	6.6	0.033	121.7 ± 40.2	110.5 ± 4.5
270	205	7.3	0.035	54.9 ± 19.8	39.1 ± 13.0

DOSE 81mg/kg.

TIME	BODY WEIGHT	LIVER WT	LW/BW	PLASMA HYDRAZINE	LIVER HYDRAZINE
10	202	6.6	0.033	305.5 ± 193.1	161.7 ± 18.9
30	210	7.2	0.034	835.6 ± 868.5*	215.4 ± 127.9
90	209	6.9	0.033	1619.4 ± 2411.9*	134.6 ± 80.9
270	205	7.6	0.037	29.7 ± 22.4	13.1 ± 2.8

Values are means of from 3 rats S.D.
* 1 individual result is very high.

TABLE 2

RATIO OF LEVELS OF HYDRAZINE IN PLASMA TO LIVER AFTER VARIOUS DOSES

	DOSE LEVEL mg/kg; oral			
	PLASMA: LIVER			
TIME AFTER DOSING Minutes	3	9	27	81
10	0.67	0.3	1.46	1.21
30	0.63	1.34	2.76	1.60
90	0.74	1.54	1.10	1.69
270	1.21	0.98	1.40	2.27

TABLE 3.

MEAN BODY WT, URINE OUTPUT AND WATER INTAKE FOR RATS EXPOSED TO HYDRAZINE AND MONITORED FOR 4 DAYS.

DAY 0		DAY 1		DAY 2		DAY 3		DAY 4	
BW	DOSE	BW	U/W	BW	U/W	BW	U/W	BW	U/W
223	0	241	12/37	249	11/30	258	9/30	265	9/28
224	3mg/kg	242	13/38	253	13/34	263	12/34	274	9/27
218	9mg/kg	231	11/31	238	10/31	246	8/29	246	7/26
225	27mg/kg	232	15/29	244	11/34	254	8/33	259	7/26
223	81mg/kg	221	44/47	218	16/22	215	10/16	209	10/10

BW = Body Weight

U/W = Urine volume/water intake.

Rats dosed orally with hydrazine in distilled water. Day 0 is day prior to dosing.

TABLE 4.

MEAN ORGAN WEIGHTS EXPRESSED AS A RATIO TO BODY WEIGHT IN RATS 4 DAYS AFTER DOSING WITH HYDRAZINE ORALLY.

DOSE mg/kg	B W	LIVER WT/BW	TESTIS WT/BW	KIDNEY WT/BW	SPLEEN WT/BW	HEART WT/BW
0	265	0.041	0.011	0.0076	0.0032	0.0033
3	274	0.043	0.0098	0.0078	C.0031	0.0038
9	246	0.044	0.011	0.0078	0.0027	0.0038
27	259	0.047*	0.011	0.0087	0.0031	0.0035
81	209	0.042	0.012	0.0100	0.0025	0.0035

BW = Final Body Weight
Results are means from 3 rats S.D.
* $p<0.001$ compared with control.

TABLE 5

EXCRETION OF HYDRAZINE IN THE URINE OF RATS AFTER VARIOUS DOSES.

DOSE mg/kg	HYDRAZINE % DOSE	ACETYLHYDRAZINE	ACHZ: HZ
3	71±52	11±5	0.13
9	85±28	8±1	0.10
27	41±10	4±1	0.09
81	35±7	2±0.8	0.06

Values are means from 3 rats; excretion in urine 0-24 h after dosing.

TABLE 6

LIVER AND PLASMA CONCENTRATIONS OF HYDRAZINE

	HYDRAZINE DOSE mmol/kg		
	2.5		1.88
TIME AFTER DOSE	4h	24h	24h
	HYDRAZINE CONCENTRATIONS mmol/ml or g		
LIVER	81.4±11.3	11.0±5.4	2.2±0.75
PLASMA	51.4±1.9	1.9±0.3	0.5±0.1

Rats were dosed with ^{15}N hydrazine sulphate.
Results are means ±S.E. from 3 rats.

TABLE 7

METABOLISM OF HYDRAZINE IN MICROSOMES IN VITRO

TOTAL HYDRAZINE METABOLISM
micromoles. (% control)

CONDITION	n	TIME 15 min	60 min
Complete System	4	1.48±0.32	3.05±0.25
CO/O	1	0.20 (8%)	0.32 (12%)
N_2	1	0.12 (12%)	0.17 (3%)
Piperonyl Butoxide	2	0.23 (11%)	1.34 (51%)
Metyrapone	1	1.55 (78%)	2.04 (88%)
Methimazole	3	0.76 (60%)	2.42 (80%)

Results are means of triplicate determinations, repeated n times.
Hydrazine disappearance at 0° in the presence of microsomes and a complete NADPH regenerating system have been substracted from each value.

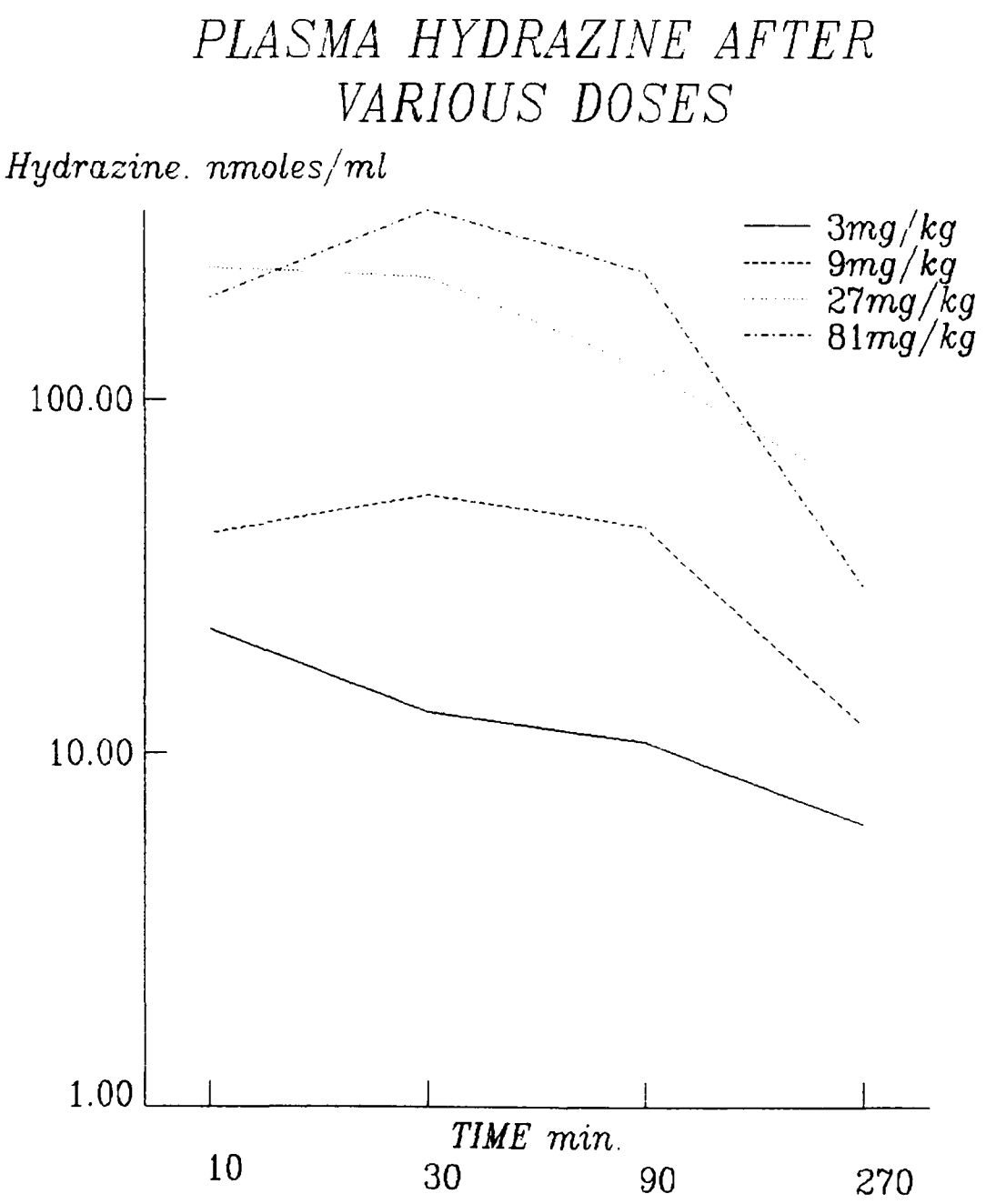
PLASMA HYDRAZINE AFTER VARIOUS DOSES
Hydrazine. nmoles/ml
3mg/kg
9mg/kg
27mg/kg
81mg/kg
100.00
10.00
1.00
TIME min.
10
30
90
270

FIGURE 1

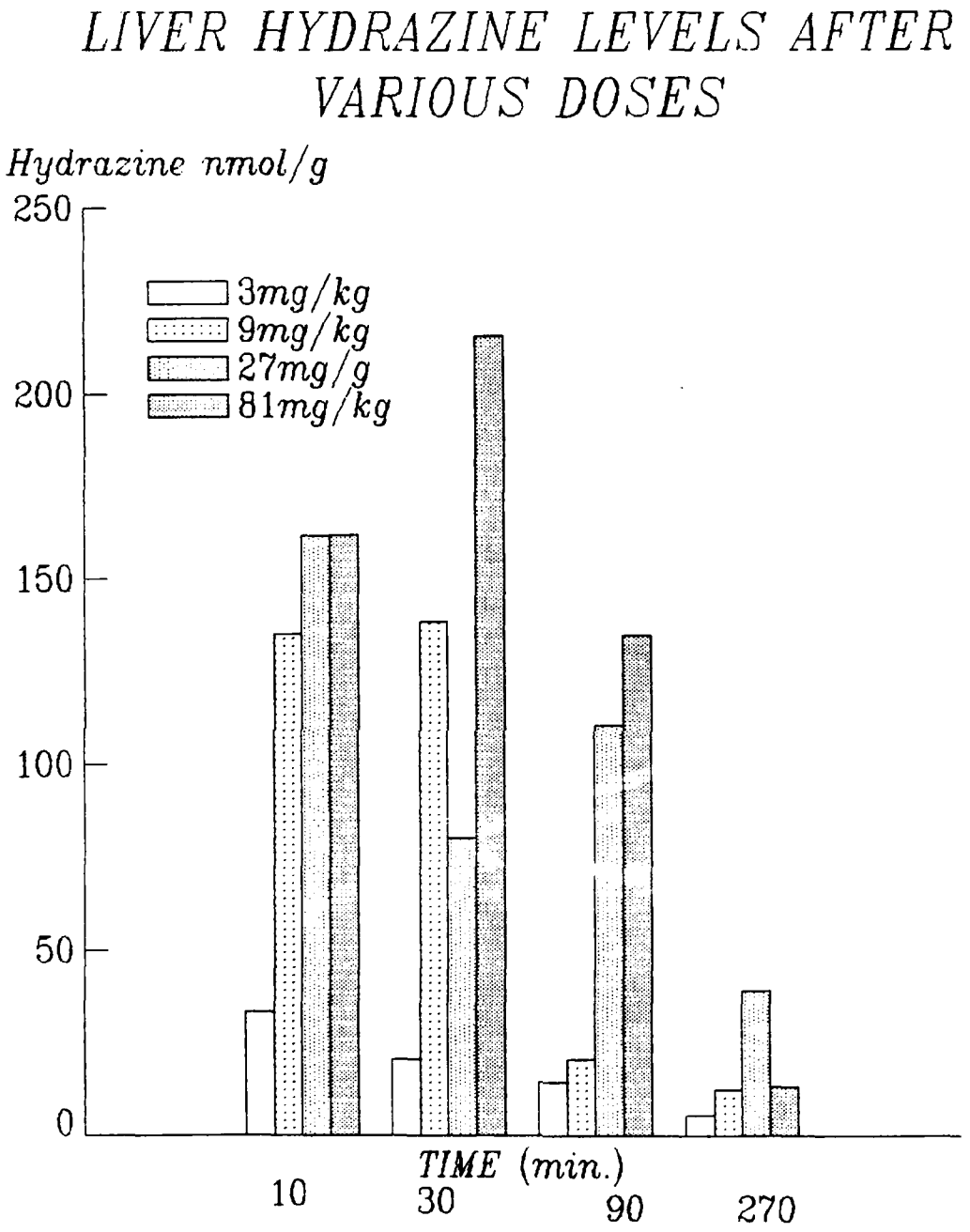
LIVER HYDRAZINE LEVELS AFTER VARIOUS DOSES
Hydrazine nmol/g
250
200
150
100
50
0
3mg/kg
9mg/kg
27mg/g
81mg/kg
TIME (min.)
10
30
90
270

FIGURE 2

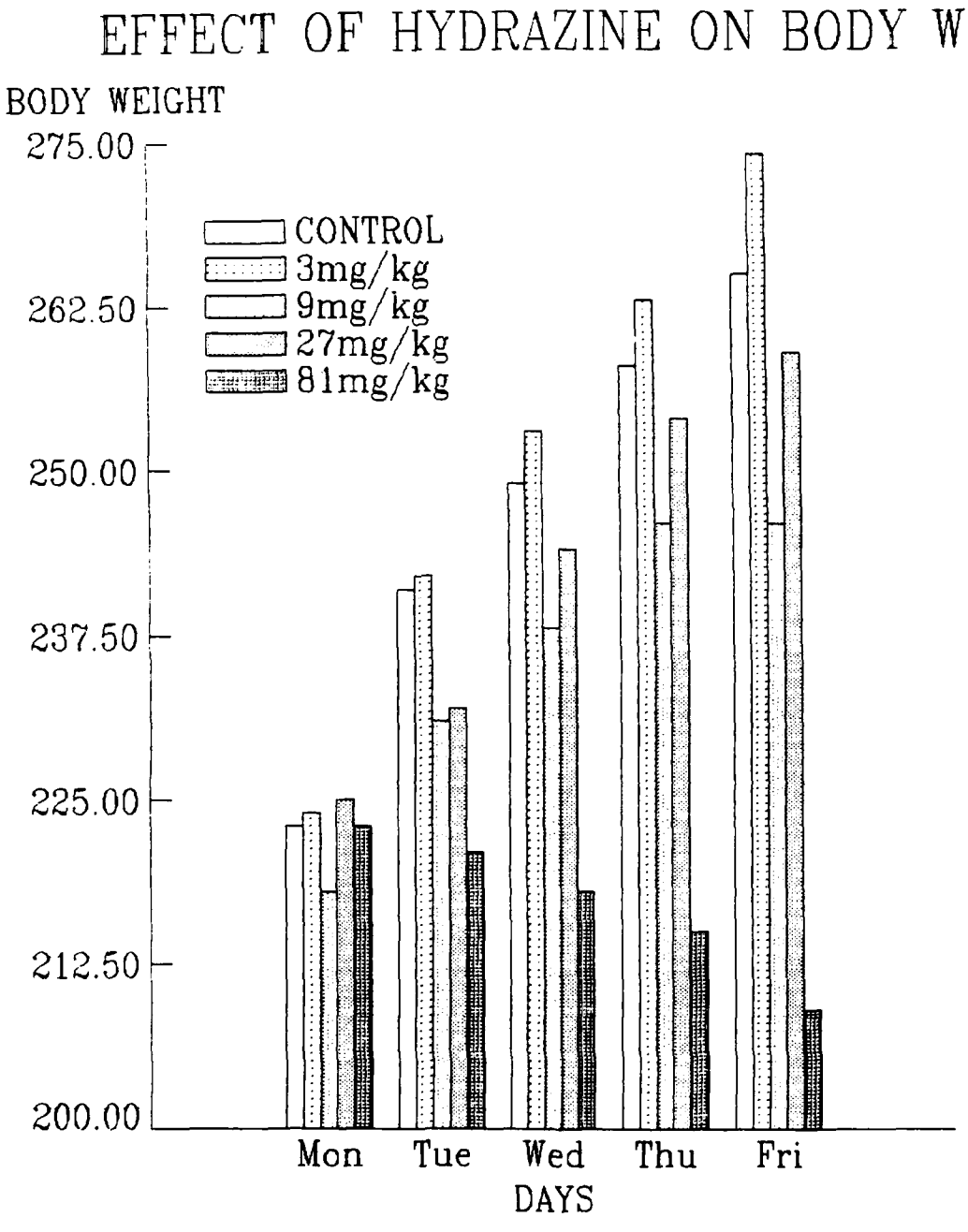
EFFECT OF HYDRAZINE ON BODY WT
BODY WEIGHT
275.00
262.50
250.00
237.50
225.00
212.50
200.00
CONTROL
3mg/kg
9mg/kg
27mg/kg
81mg/kg
Mon
Tue
Wed
Thu
Fri
DAYS

FIGURE 3

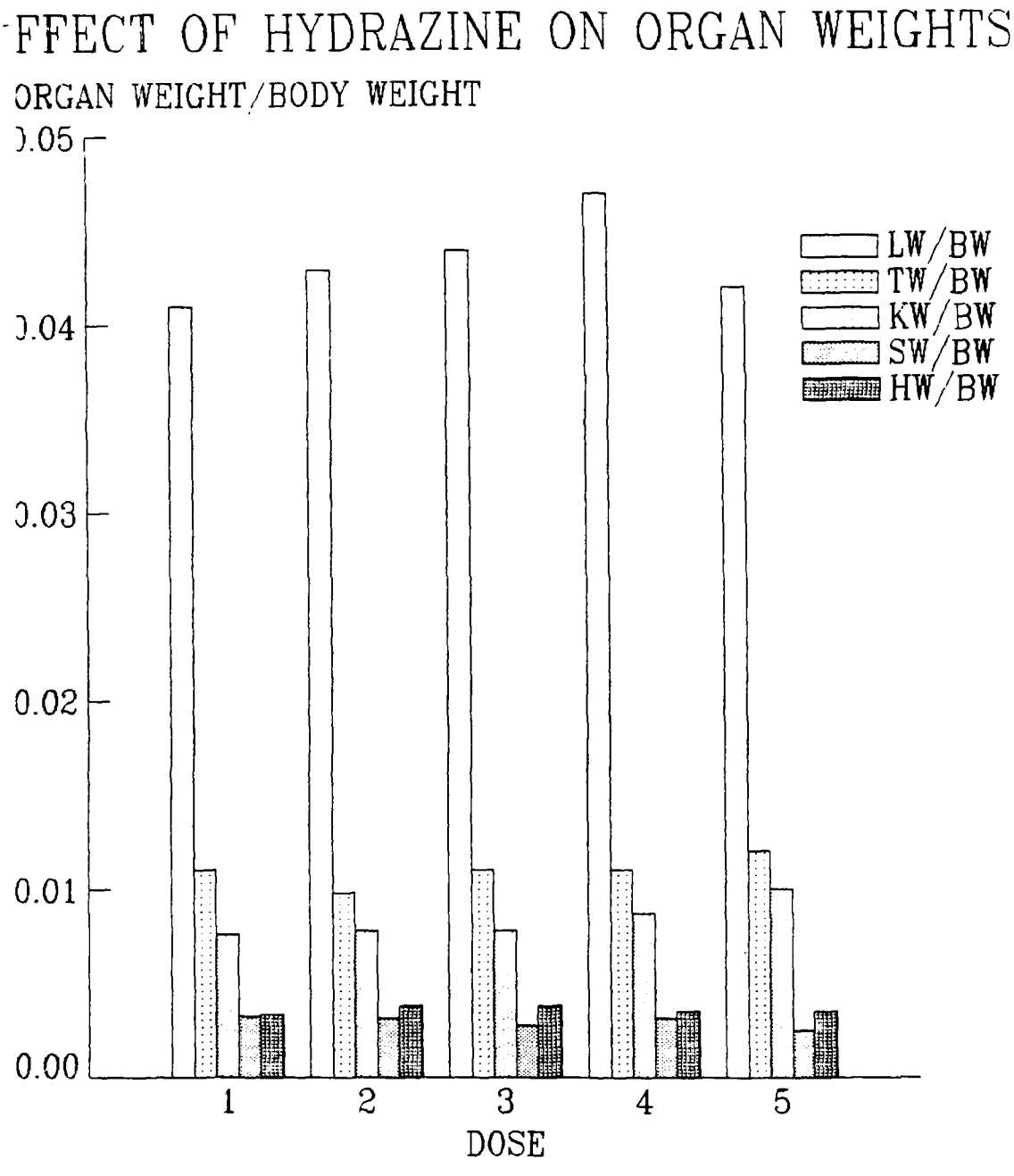
FFECT OF HYDRAZINE ON ORGAN WEIGHTS
ORGAN WEIGHT/BODY WEIGHT
0.05
0.04
0.03
0.02
0.01
0.00
LW/BW
TW/BW
KW/BW
SW/BW
HW/BW
1
2
3
4
5
DOSE

FIGURE 4

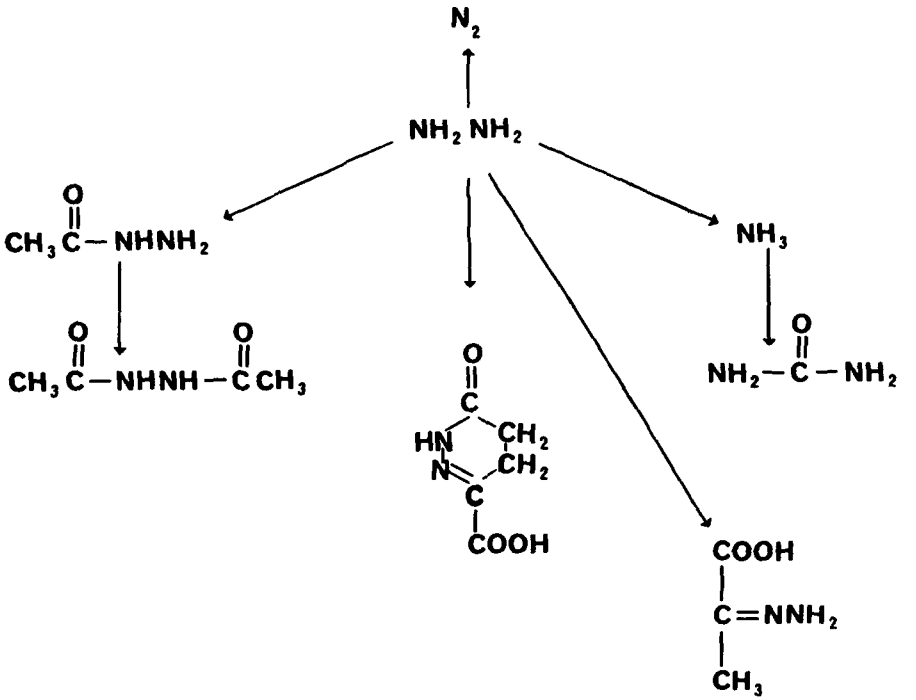
N2
NH2 NH2
CH3C(=O)NHNH2
CH3C(=O)NHNHC(=O)CH3
NH3
NH2C(=O)NH2
HN
N
CH2
CH2
C(=O)
C
COOH
COOH
C=NNH2
CH3

FIGURE 5

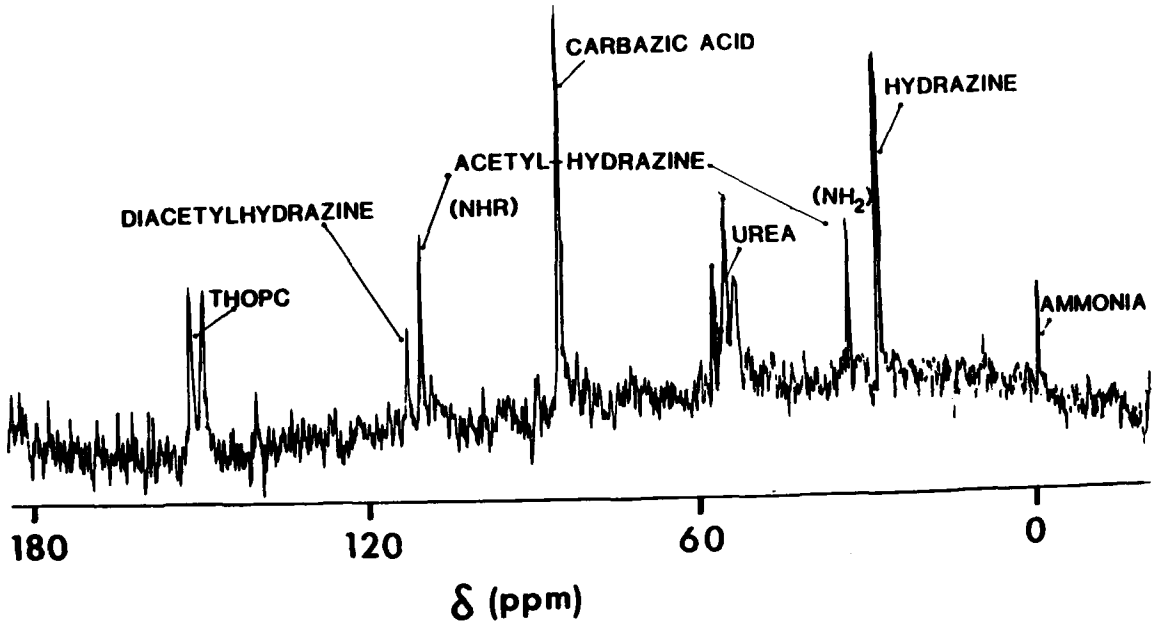
CARBAZIC ACID
HYDRAZINE
ACETYL-HYDRAZINE
DIACETYLHYDRAZINE
(NHR)
(NH2)
UREA
THOPC
AMMONIA
180
120
60
0
δ (ppm)

FIGURE 6

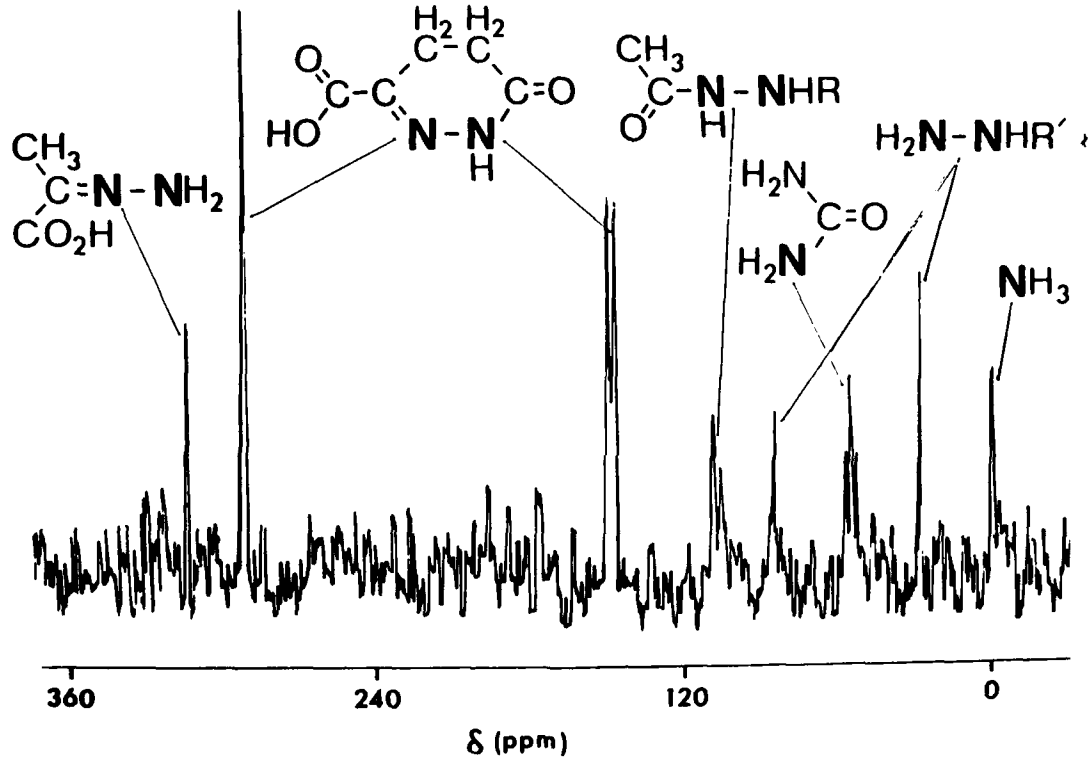
CH3
C=N-NH2
CO2H
H2 H2
C-C
O=C-C
HO
C=O
N-N
H
CH3
C-N-NHR
O
H
H2N-NHR'
H2N
C=O
H2N
NH3
360
240
120
0
δ (ppm)

FIGURE 7

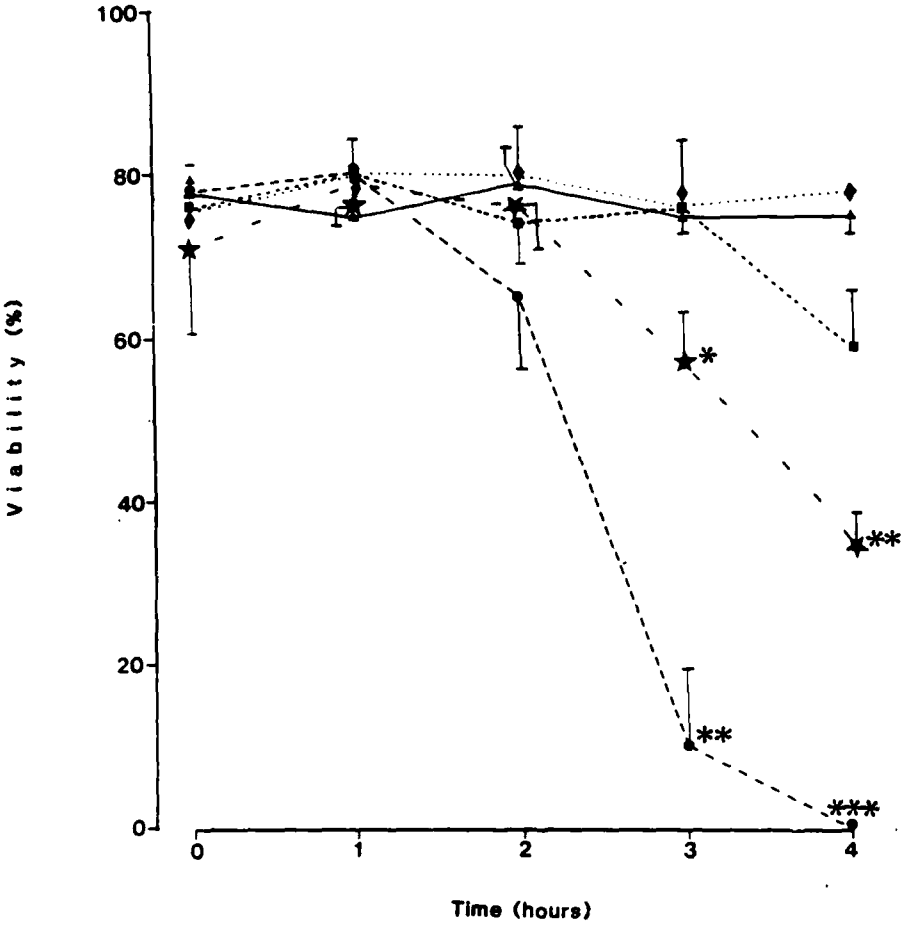

FIGURE 8

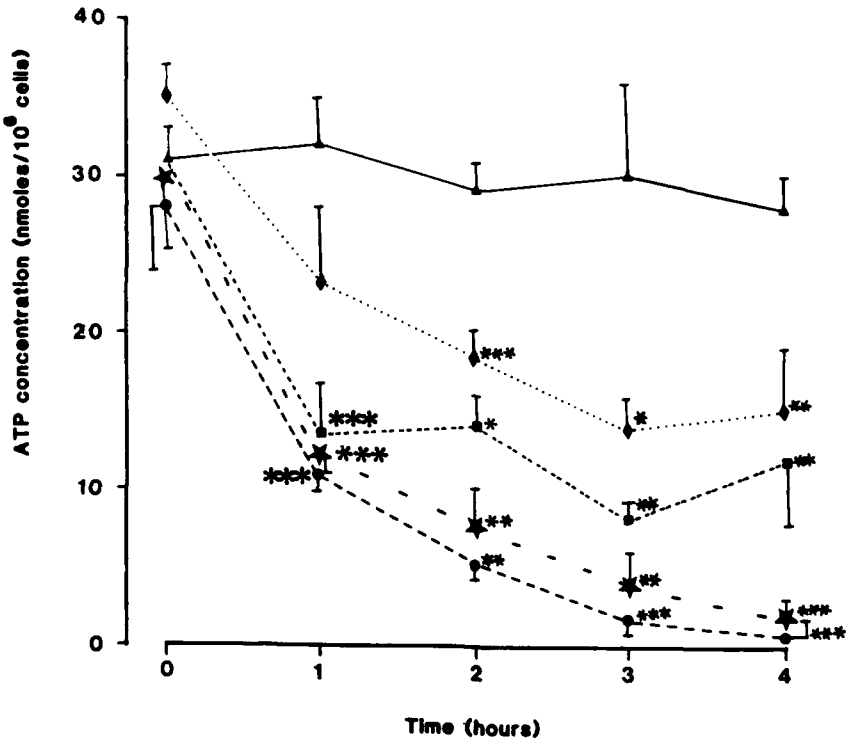
ATP concentration (nmoles/10^6 cells)
40
30
20
10
0
0
1
2
3
4
Time (hours)

FIGURE 9

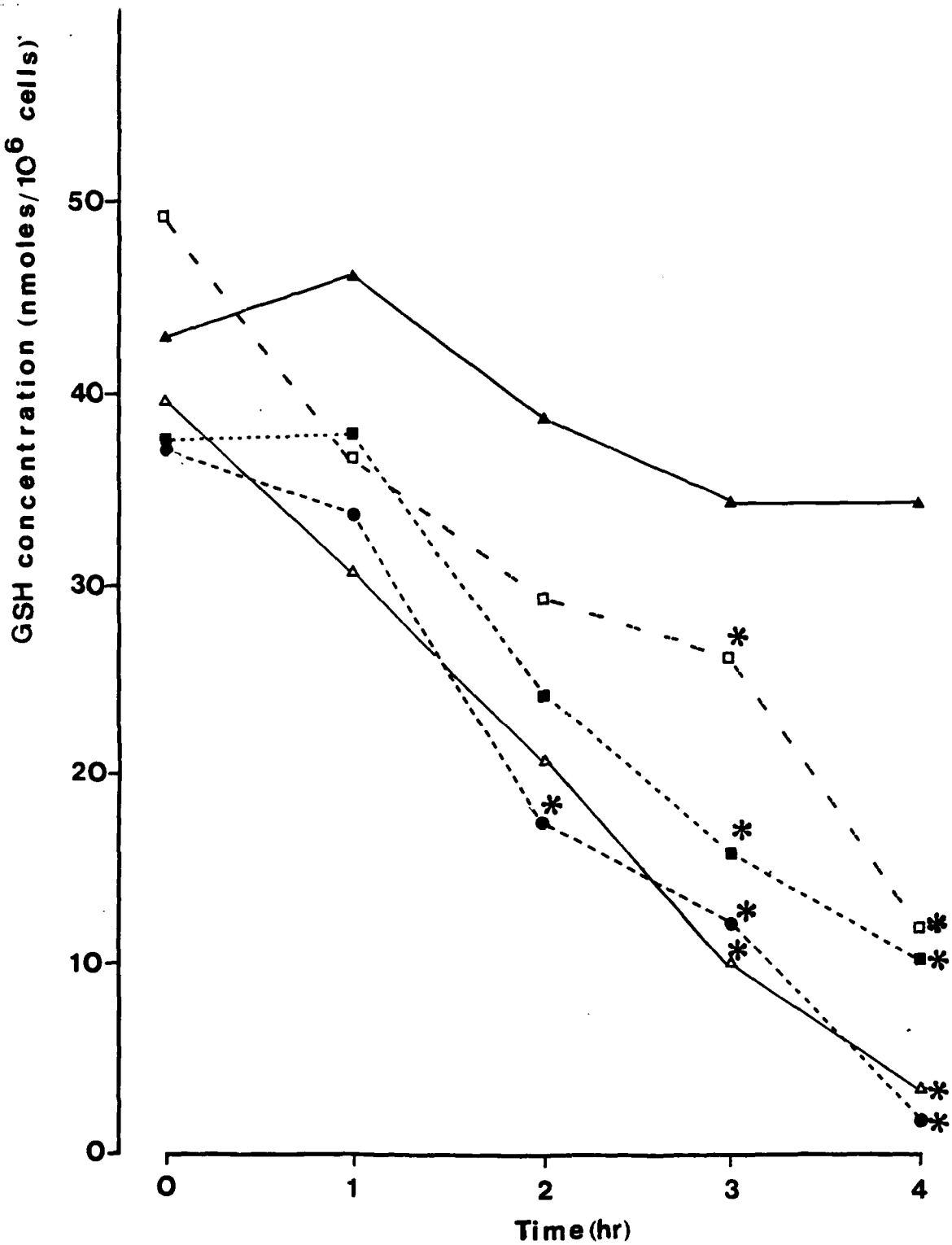
GSH concentration (nmoles/10^6 cells)
50
40
30
20
10
0
0
1
2
3
4
Time (hr)

FIGURE 10

The DMBA Colorimetric Method for the Determination of Hydrazine in Microsomal Suspensions

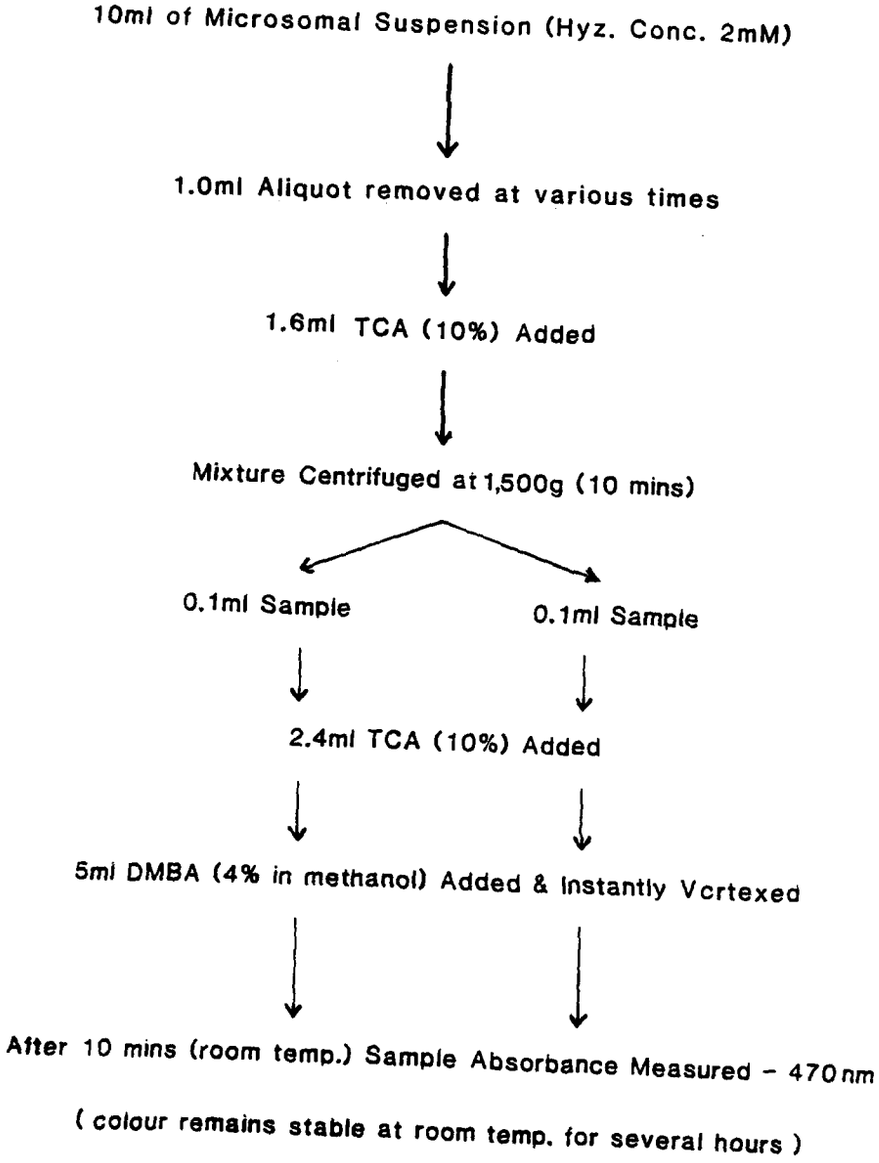

FIGURE 11